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Tools and methods for providing assurance of clonality for legacy cell lines

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Providing Assurance of Clonality for Legacy Cell Lines

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Summary

Over the last several years demonstration of cell line clonality has been a topic of many industry and regulatory presentations and papers. The rationale behind characterizing clonality stems from the expectation that bioprocesses must produce a consistent biologic. A clonally-derived cell line should help ensure a consistent product profile. However, increased attention on this aspect of cell line development has resulted in industry questioning of the relative importance of clonality in the larger context of product quality and process consistency testing (Frye et al., 2016). It is the position of the current authors that assurance of a consistent biologic is best addressed by the totality of data including process and product consistency, as monoclonality is only one portion of the entire control strategy. Though debate continues on the relevance of a clonal population to the consistency of a product, many have implemented procedures and technology intended to achieve acceptable assurance of clonality for products entering clinical development today. This move has strengthened the industry as a whole, but many sponsors still face a challenge with older cell lines created in a manner that did not meet the current set definition of clonality, which we refer to as "legacy cell lines" in this article. In this poster we, members of the IQ Consortium working group on clonality, present our position on genetic testing of legacy cell lines to characterize clonal origin, methods that could be useful for genetic analysis, and case studies that highlight the pros and cons of such testing in light of its relative importance to regulatory filings for biologics production.

Introduction and Team Position

Regulatory guidance (ICH Q5D) instructs cloning the cell substrate "from a single cell progenitor" during cell line development. Presentations from FDA colleagues and industry experience over the last several years have established an expectation for sponsors to provide high assurance of clonality (Kennett, 2014; Novak, 2017; Welch, 2017). The FDA has recommended that two-rounds of limiting dilution cloning (LDC) at sufficiently low seeding densities (≤0.5 cells/well) provides acceptable probability that a cell line is clonal. More recently, one-round of cloning through FACS or LDC with sufficient supporting justification, such as use of imaging technology, has provided acceptable assurance of clonality when using validated methods. However, some ongoing clinical programs employ legacy cell lines that were created before the industry had such practices and methods in place, and may not satisfy current regulatory expectations for clonality.

The choice of if or when to implement additional work to provide supplemental assurance of clonality is something each sponsor must evaluate individually based on available process and product data, product stage, and individual experience. The typical stages in which data are obtained for justifying that a cell line is appropriate for commercial production are provided in Figure 1. Ultimately, the BLA will include data from extensive process characterization studies performed to demonstrate consistent product quality and cell culture performance from qualified scale-down, pilot and commercial scales, as well as cell line genetic characterization studies to show stable transgene integration profiles. These data should suffice for approval, but are typically not available until late in product development and present challenges during earlier clinical development stages to properly mitigate filing risk associated with a putative non-clonal bank.

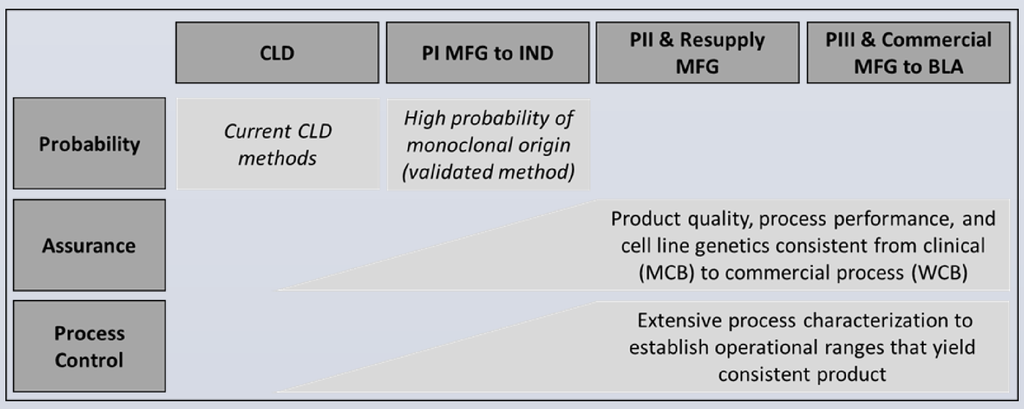

Figure 1. Typical Approach to Supporting Clonality

The FDA has indicated a willingness to accept different types of genetic data as additional assurance of clonality, including characterizing individual subclones from the MCB (Welch, 2017). A number of innovative technologies and approaches to providing additional assurance of clonality are presented in this article. Although new technologies provide an ability to analyze a cell line's genetic profile in detail, the consensus of these authors is that acceptance of these approaches by regulatory agencies is still unknown. This uncertainty is outlined conceptually in Figure 2, showing an example approach to provide additional assurance of clonality that could avoid a request for implementing additional process controls. However, it is the experience of some of the authors that providing additional assurance of clonality has not been acceptable to preclude the requirement for augmented control strategy, even when consistent genetic profiles indicate a monoclonal cell origin.

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We propose the purpose of performing additional assurance experiments to demonstrate monoclonal origin should be eliminating the need for additional process control. This position, we feel, needs to be clearly addressed by regulatory authorities so that the supplemental work is not done in vain.

Figure 2: Decision tree for implementing additional genetic testing as assurance of clonality

The ultimate requirement for all commercial cell lines, regardless of Cell line Development history, is demonstration of consistent process performance and product quality during process characterization (Process Control) and conformance (Assurance). These data are not typically available at early clinical stage to support the consistency of legacy cell lines. As such, some legacy cell lines may receive IND non-hold questions regarding clonality and a request to provide additional assurance of clonal origin (Additional Assurance). The authors propose that if additional genetic testing is performed and provides assurance of clonal origin then no further proof of clonality should be required (dark grey YES arrow).

If genetic testing does not support clonality or is inconclusive then augmented control strategies may be required (dark grey NO arrow), depending on the totality of data, including product quality, process performance and stability. Sponsors may choose not to perform additional genetic tests (dashed arrows), opting to 1) proceed with standard process control strategies or, based on project status, 2) implement augmented control strategies. The expectations at this decision junction need further elucidation from health authorities as some authors have experienced requests for augmented control strategies in addition to genetic testing that could demonstrate cell line clonality.

Optional Genetic Testing as Assurance for Clonality

Method	Primary Clone	Analytical Subclones
Southern Blot	Traditionally used to compare MCB to EOP cells. Accepted methodology for BLA filing.	A single shared hybridization band in a suitable number of analytical subclones can provide assurance of clonal origin.
FISH	Karyotype analysis of individual cells from the MCB can identify unique, consistent integration sites to support clonal origin.	Not necessary as integration site consistency can be detected in MCB.
Next Generation Sequencing	Whole genome sequencing and Targeted Locus Amplification can provide detailed transgene and integration site DNA sequence. TLA provides greater sequence coverage of the targeted transgene and flanking genome and can detect low frequency gene of interest sequence variants. RNAseq can be used to validate low level sequence variants.	Unique transgene integration sites or sequence variant markers can be used as clone-specific markers for subsequent PCR-based assays. Markers identified in a suitable number of analytical subclones can provide assurance of clonal origin. These NGS methods could be performed directly on analytical subclones but are data and cost intensive compared to PCR-based assays.
PCR	Inverse and Splinkerette PCR can be used to identify transgene genomic integration fusion junctions and flanking sequences.	Genomic integration site junctions are unique identifiers that can be used for comparing a suitable number of analytical subclones to provide assurance of clonal origin.

Conclusion

We recognize that additional assurance studies do not fully mitigate risk, as genetic demonstration of clonal origin does not ensure process consistency and, conversely, non-clonal cell lines can produce consistent process/product. It is important to highlight that no one genetic technique is sufficient to demonstrate clonality conclusively due to the plasticity of the CHO cells and high rate of genetic drift. They at best provide the supporting data that the cell line was clonally-derived. The methods described herein can be useful for supporting monoclonal origin, but can also reveal genetic inconsistencies that are not easily explained. Thus, the proposed methods are useful if they provide assurance of monoclonality, and can strengthen regulatory filings, but may not change the course of development if they do not provide clear assurance of clonality, depending on demonstration of process consistency.

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